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PB-266 311

Effects of Inorganic Complexing on the Toxicity of Copper to 'Daphnia magna'

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26 Sep 76

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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PB266311

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1. REPORT NO.	2.	3.
EPA-600/J-77-001	JOURNAL ARTICLE	PB 266 311

4. TITLE AND SUBTITLE
EFFECTS OF INORGANIC COMPLEXING ON THE TOXICITY OF COPPER TO DAPHNIA MAGNA

5. REPORT DATE

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
Andrew, R.W., K.E. Biesinger, and G.E. Glass

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
U.S. Environmental Protection Agency
Environmental Research Laboratory-Duluth
6201 Congdon Boulevard
Duluth, MN 55804

10. PROGRAM ELEMENT NO.
1BA608

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
SAME

The report includes the

13. TYPE OF REPORT AND PERIOD COVERED
Scientific Journal Publ.

14. SPONSORING AGENCY CODE
EPA/600/03

15. SUPPLEMENTARY NOTES
Published in Water Research 11:309-315 (1977)

16. ABSTRACT

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U. S. DEPARTMENT OF COMMERCE
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17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Bioassay Toxicity Copper (0702) Water pollution Chelation	Daphnia magna Pollution effects	06T 07B

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	9
	20. SECURITY CLASS *(This page)*	22. PRICE
	UNCLASSIFIED	A02-A01

EPA Form 2220-1 (9-73)

EPA-600/J-77-001
JOURNAL ARTICLE

Water Research Vol. 11, pp. 309 to 315. Pergamon Press 1977. Printed in Great Britain.

EFFECTS OF INORGANIC COMPLEXING ON THE TOXICITY OF COPPER TO *DAPHNIA MAGNA*

R. W. ANDREW, K. E. BIESINGER and G. E. GLASS

U.S. Environmental Protection Agency, Environmental Research Laboratory-Duluth, 6201 Congdon Boulevard, Duluth, Minnesota 55804, U.S.A.

(*Received* 26 *September* 1976)

Abstract—Effects of carbonate–bicarbonate, orthophosphate, and pyrophosphate on the toxicity of copper (II) to *Daphnia magna* were studied at constant pH and total hardness. Mortality rates and reciprocal survival times were directly correlated with cupric (Cu^{2+}) and copper hydroxy ($Cu(OH)n$) ion activities as determined by equilibrium calculations. Toxicity was negatively related to activities of soluble copper carbonate ($CuCO_3$) and other complexes, and was found to be independent of dissolved copper or total copper concentrations.

INTRODUCTION

Testing toxicity of the heavy or transition metal salts with aquatic organisms presents a difficult bioassay situation. Since these metals when dissolved or suspended in water react chemically or are adsorbed by particulate matter to some degree, the active concentration to which the animal is exposed and in many cases the chemical form of the metal are not clearly defined. A major factor complicating studies on the toxicity of the metals is the complexity of their aqueous chemistry. In natural waters the metals readily form stable hydroxy or carbonate complexes or both, and only a fraction of the total concentration exists as the cationic or aquo form (Stumm & Morgan, 1970). In most acute toxicity tests metal solubility is limited by precipitation of the common hydroxides, carbonates, or phosphates, or a combination of these. Historically, metal toxicity tests in natural waters are noted for their variety of numerical results and biological interpretations, usually with little or no chemically relevant data.

An additional difficulty in interpreting such tests is that, since the animal lives in the test solution, any chemical or physical interactions that take place may affect the animal physiologically, in addition to the direct toxic response that is to be measured. Small changes in dissolved oxygen and CO_2 content, pH, alkalinity, and hardness of the test water alter both the aqueous chemistry of the toxicant and the animal's physiological response to that toxicant (Jones, 1939). Although variations in metal toxicity have long been recognized as generally related to water chemistry, few concerted efforts have been made to determine the basic chemical relationships affecting such differences. Reporting of analytical determinations of "dissolved" or ionic concentrations is a relatively recent development in the aquatic toxicology literature (see, for example, Cairns *et al.*, 1971; Zitko *et al.*, 1973; Shaw & Brown, 1974). Interpretation of chemical effects in the past has been limited mainly to observations accompanying gross changes in the character of the test waters.

Much of the early work on effects of chemical variables on metal toxicity dealt with hardness or the principal hardness cations, calcium and magnesium (Skidmore, 1964). The physiological basis of such cationic antagonism is generally accepted. Tabata (1969), Stiff (1971), and Fagenkopf *et al.* (1974) have indicated that the bicarbonate alkalinity usually associated with hard waters may also reduce toxicity through formation on nontoxic carbonate complexes or precipitates.

Mount (1966), Steeman Nielsen *et al.* (1969), Mancy & Allen (1976), and others have investigated the effect of changes in pH on toxicity of the metals and have concluded that toxicity increases with increasing pH. However, a recent paper by Shaw & Brown (1974) indicates that toxicity of copper to trout is the same at pH 6.5 as at 7.5. Tabata (1969), Cairns *et al.* (1971), and Davies & Everhart (1973) indicate that precipitates formed at high pH are nontoxic except when ingested by filter-feeding organisms. Conflicting interpretations of pH effects continue because of the difficulty of separating physiological from external chemical effects.

Recent investigations of toxicity in the presence of added complexing agents indicate that formation of either inorganic or organic complexes greatly reduces metal toxicity. Nishikawa & Tabata (1969) have observed that the reduction in toxicity is related to the stability constants of the metal complexes formed. Grande (1966), Sprague (1968), Tabata & Nishikawa (1969), and Biesinger *et al.* (1973) have shown that zinc and copper solutions with an excess of the strong chelating agents EDTA or NTA are much less toxic to fish and aquatic invertebrates. Similarly, thiosulfate (Nishikawa & Tabata, 1969), citrate (Carr, 1967), glycine (Shaw, 1969), and humic acids (Zitko *et al.*, 1973) reduce toxicity in proportion to the concentration of the added chelant. Doudoroff *et al.* (1966) showed

that the toxicities of complex metal–cyanide mixtures could be predicted on the basis of individual ion toxicities and molecular hydrogen–cyanide concentrations calculated from chemical equilibrium equations. The metal cyanide complexes also were shown to be essentially nontoxic.

In this study, three toxicity experiments with *Daphnia* were conducted. The first was designed to test the overall effect of fixed additions of bicarbonate, orthophosphate, and pyrophosphate on copper solubility, ionic distribution and toxicity. In the second and third tests, a single total copper concentration was tested with a range of bicarbonate and pyrophosphate concentrations, respectively, in order to determine relative toxicities of cupric ion and its complexes. Equilibrium calculations were used to determine the aqueous copper species present, and toxicity was correlated with ionic and molecular species activities. Total hardness (Ca + Mg) and pH were held constant to eliminate their effects on toxicity.

MATERIALS AND METHODS

Lake Superior water, used as the dilution water for all experimental work, has a total hardness of ~ 45 mg l^{-1}, alkalinity of ~ 42 mg l^{-1}, and a pH from 7.4 to 8.2 (these and other chemical characteristics are summarized in Biesinger & Christensen, 1972).

Solutions of reagent grade (ACS) chemicals were prepared in Lake Superior water to maintain a constant ionic background in all test solutions. Copper solutions were prepared from ultrapure copper(II) perchlorate (G. F. Smith Chem. Co.*). Dilutions of stock solutions were prepared in airtight polyethylene bottles and allowed to equilibrate for 24 h before testing. The pH was adjusted in each experiment by regulation of CO_2–air mixtures bubbled through the solutions to maintain the total carbonate alkalinity constant.

Total copper concentrations were measured by atomic absorption spectrometry after extraction of the cupferron complex in methyl isobutyl ketone at neutral pH (Arthur & Leonard, 1970). Dissolved copper was measured in a similar fashion after the solution was filtered through a 0.45 μm membrane filter under vacuum. The pH of all solutions was measured at the beginning and the end of the experiments. Results in most cases varied by less than 0.2 pH unit. Terminal values were used for all calculations.

Dissolved oxygen and total alkalinity were measured by procedures outlined by the American Public Health Association (1971). Total alkalinity, with appropriate deduction of added phosphate alkalinity, and pH were used to estimate the carbonate and bicarbonate concentrations in each solution. The equilibrium concentrations of the major aqueous species of copper were calculated using the computer program developed by Morel & Morgan (1972) and stability constants from the compilations of Sillén & Martell (1971). Equilibrium calculations were based on measured dissolved copper concentrations to minimize the effects of precipitation on toxicity correlations.

Initial calculations included consideration of all protonated, hydroxylated, and polydentate copper complexes of carbonate, orthophosphate, pyrophosphate, chloride, and sulfate as listed by Sillén & Martell (1971). Very weak complexes, i.e., those with stabilities resulting in equilibrium concentrations less than 0.1% of the Cu^{2+} ion at the observed pH were dropped from final tabulations. Potential biological effects of these latter forms appear from the results to be relatively insignificant.

Daphnia magna from a clone maintained at the Environmental Research Laboratory-Duluth was used for all tests. Ten animals, 0–24-h-old, were placed into each of duplicate 300-ml BOD bottles containing test solutions. The bottles were filled with solutions to exclude air and were stoppered throughout the test (2–3 days). Dissolved oxygen concentrations remained near saturation throughout the tests. The bottles were randomized and placed in a constant temperature room at 18° ± 1°C. A combination of Duro-Test (Optima FS) and wide spectrum Gro-Lux fluorescent bulbs provided a light intensity of ~ 115 lumens.

Daphnids were observed at 30-min intervals during the first 4–6 h and hourly up to 24 h. Additional observations were made at 4–6 h intervals for the remainder of each test. Observations were continued until all animals were dead or until the end of 72 h. Complete immobilization of the daphnids (as noted by slow turning of the bottles) was used as the biological endpoint. The repeated observations allowed correction of earlier observations when occasional animals revived. Animals were not removed until the test was ended and chemical measurements had been made.

Percentage survival of the organisms in each of the chambers was evaluated as a function of time; results from duplicate chambers were combined for statistical calculations. The logarithm of percentage survival vs. time was evaluated by linear least squares regression techniques and was used to calculate the linear mortality rate (Chen & Selleck, 1969) and the median survival time. Reciprocal survival time as used in the text and figures indicates the reciprocal ($1/T$) of the median survival time as determined above.

Linear least squares and multiple linear regression techniques (Steel & Torrie, 1960) were also used to ascertain correlations of toxicity with chemical variables.

RESULTS

Effect of carbonate–bicarbonate, orthophosphate and pyrophosphate on copper toxicity

Mortality rates and median survival times of *Daphnia magna* in Lake Superior water, and with additions

* The U.S. Environmental Protection Agency neither recommends nor endorses any commercial product; trade names are used only for identification.

Table 1. Copper solubility and toxicity to *Daphnia magna*, showing effects of bicarbonate, ortho-, and pyrophosphate additions to Lake Superior water (pH 7.85–8.05)

Nominal total Cu (μM)	Measured dissolved Cu (μM)	*Daphnia* toxicity: Mortality rate*	*Daphnia* toxicity: Median survival time (min)
	Lake Superior water only		
Control	~0.02	0.0	>2,880
0.20	0.20	0.2	1,740
0.32	0.33	1.8	480
0.51	0.41	1.7	372
0.80	0.61	7.7	168
1.27	1.02	5.8	180
2.01	1.54	12	84
	Lake Superior water + 48 μM $NaHCO_3$		
Contol	~0.02	0.0	>2,880
0.80	0.60	2.7	346
1.27	0.98	6.8	120
2.01	1.45	9.7	~84
	Lake Superior water + 210 μM Na_2HPO_4		
Contol	~0.02	0.0	>2,880
0.80	0.54	0.8	618
1.27	0.76	1.1	486
2.01	1.07	1.8	316
	Lake Superior water + 110 μM $Na_4P_2O_7$		
Control	~0.02	0.0	>2,880
0.80	0.77	0.0	>2,880
1.27	1.24	0.4	1,092
2.01	1.95	0.0	>2,880

* Mortality rate = $10^3 \cdot \Delta\log(f) \cdot \Delta t^{-1}$, where f = fraction surviving at time t of exposure.

of bicarbonate, ortho- and pyrophosphate are shown in Table 1. Toxicity of copper increased roughly in proportion to nominal copper and dissolved copper concentrations in lake water, or with either bicarbonate or orthophosphate added. Toxicity at the same nominal copper concentrations varied widely with the three additions; much lower overall toxicity was observed in the presence of pyrophosphate.

Addition of orthophosphate resulted in decreased solubility of copper and a corresponding decrease in copper toxicity. Pyrophosphate, however, increased copper solubility but resulted in a nearly complete removal of toxicity. Because of these effects no consistent relationship was found between copper toxicity and total copper or dissolved copper measurements in the presence of the various complexing agents.

Calculated ionic activities and molecular concentrations of the major copper species in each of the

Table 2. Calculated copper species activities showing effects of bicarbonate, ortho-, and pyrophosphate additions to Lake Superior water (pH 7.85–8.05)

Measured dissolved Cu (μM)*	Calculated activity of copper species (μM): Cu^{2+}	$CuOH^+$	$Cu(OH)_2^0$	$CuCO_3$	$Cu(CO_3)_2^{2-}$	Other forms (total)
			Lake Superior water only			
~0.02	0.001	0.001	—	~0.02	—†	—
0.20	0.011	0.009	—	0.18	0.001	—
0.33	0.016	0.015	—	0.30	0.002	—
0.41	0.020	0.019	—	0.36	0.002	—
0.61	0.031	0.028	—	0.54	0.003	—
1.02	0.054	0.047	0.001	0.89	0.005	—
1.54	0.076	0.071	0.002	1.35	0.008	—
			Lake Superior water + 48 μM $NaHCO_3$			
~0.02	0.001	0.001	—	~0.02	—	—
0.60	0.029	0.026	—	0.54	0.003	—
0.98	0.047	0.042	0.001	0.87	0.005	—
1.45	0.050	0.063	0.001	1.29	0.079	—
			Lake Superior water 210 μM Na_2HPO_4			
~0.02	0.001	0.001	—	~0.01	—	0.001 $CuHPO_4$
0.54	0.017	0.024	—	0.46	0.004	0.030 $CuHPO_4$
0.76	0.022	0.034	—	0.65	0.006	0.038 $CuHPO_4$
1.07	0.031	0.048	0.001	0.91	0.009	0.056 $CuHPO_4$
			Lake Superior + 110 μM $Na_4P_2O_7$			
~0.02	—	—	—	—	—	~0.02 $Cu(P_2O_7)^{-}$
0.77	0.002	0.002	—	0.035	—	0.61 $Cu(P_2O_7)$
1.24	0.002	0.003	—	0.059	—	0.99 $Cu(P_2O_7)$
1.95	0.003	0.005	—	0.105	—	1.54 $Cu(P_2O_7)$

* 1 μM = 63.5 μg Cu l^{-1}.
† Dash indicates less than 5×10^{-10} M.

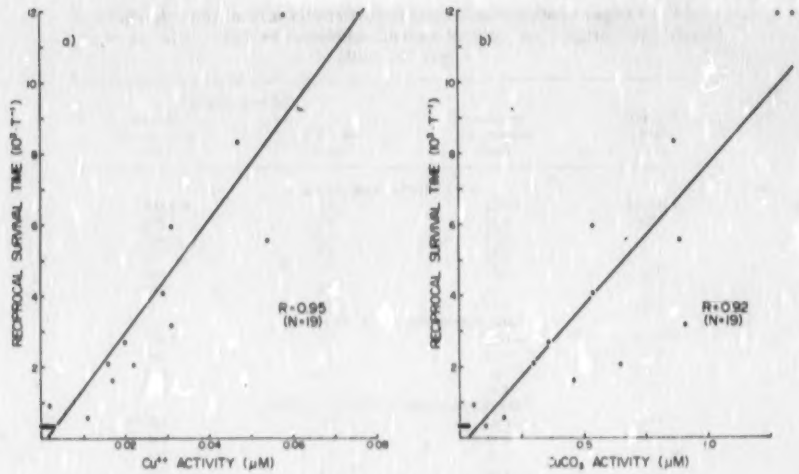

Fig. 1. Correlation of reciprocal survival times of *Daphnia* with calculated (a) cupric ion (Cu^{2+}) activity, and (b) copper carbonate ($CuCO_3$) activity in Lake Superior water with additions, pH 7·95 ± 0.1. The copper species concentrations in these solutions are highly cross-correlated.

solutions are shown in Table 2.* Equilibrium calculations showed that most of the dissolved copper in lake water and with bicarbonate added was present as the copper carbonate complex ($CuCO_3$). Only a small percentage of the total copper added remained as the free cupric ion (Cu^{2+}) at the pH of the solutions tested (7.95 ± 0.10). Addition of orthophosphate while limiting copper solubility, also shifted concentrations of the other dissolved copper species through formation of dissolved $CuHPO_4$. Addition of pyrophosphate, a moderately strong complexing agent ($pK_1 \simeq 8.8$), resulted in conversion of much of the copper added, to soluble $CuP_2O_7^{2-}$. Pyrophosphate reduced the cupric ion activity to less than 3% of that occurring in lake water with the same total copper concentration.

Statistical comparison of toxicity results (Table 1) with copper species activities (Table 2) showed that both mortality rate and the reciprocal of median survival time ($1/T$) were linearly correlated ($r = 0.70$–0.95, $P = 0.01$) with activities of the five major dissolved copper species: Cu^{2+}, $CuOH^+$, $Cu_2(OH)_2^{2+}$, $CuCO_3$, and $Cu(CO_3)_2^{2-}$. The relationship of reciprocal survival time to cupric ion (Cu^{2+}) and copper carbonate ($CuCO_3$) activities is shown in Fig. 1.

However, since activities of the major copper species were not purposely varied in these tests they are highly cross-correlated; and the relationship of toxicity to individual species activities is obscured. For this reason the relationship to cupric ion and copper carbonate was investigated further as shown in the following series of tests.

Since both ortho- and pyrophosphate effectively reduced copper toxicity, there was a negative relationship of toxicity to concentrations of both types of phosphate complexes. Too few points were available for meaningful statistical comparison, however.

Relationship of copper toxicity to total carbonate concentration

Copper solubility, activities of the major copper species, and toxicity of solutions of 5 μM total copper in Lake Superior water with additions of bicarbonate (as $NaHCO_3$) are shown in Table 3. Addition of 0–10 mM bicarbonate and adjustment of pH to 7.4 ± 0.1 with CO_2 resulted in measured total alkalinities of 42 to 511 mg l-1 (as $CaCO_3$). All animals tested in controls at the highest and lowest bicarbonate concentrations (not shown) survived for the duration of the test (1600 min).

The observed mortality rates and median survival times of *Daphnia* indicated that toxicity of the solutions was greatly diminished by the addition of bicarbonate. However, added bicarbonate resulted in increased carbonate complexing and increased solubility of copper. Under these conditions, toxicity was inversely proportional to dissoved copper concentrations and to activities of the copper carbonate complexes. Toxicity, in this case, appeared to be directly related only to the Cu^{2+} and hydroxy copper activities in these solutions.

* Following lengthy discussions and comments from reviewers, the authors have concluded that usage of classical chemical (molar) units rather than the more common mg l^{-1} in tabulations and discussion of ionic activities is not only more appropriate but is crucial to an understanding of the relative chemical and biological reactivities. Also note that because of the effects of ionic strength on individual ion activity coefficients, the sum of the ionic activities is less than the dissolved copper concentration.

Table 3. Copper solubility, calculated species activities, and toxicity of 5 μM* total copper in Lake Superior water with various concentrations of total carbonate (pH 7.3–7.5)

Concentration $NaHCO_3$ added (mM)	Measured dissolved Cu (μM)	Calculated activity of copper species (μM)					*Daphnia* toxicity	
		Cu^{2+}	$CuOH^+$	$Cu_2(OH)_2^{2+}$	$CuCO_3$	$Cu(CO_3)_2^{2-}$	Mortality rate†	Median survival time (min)
None	3.38	0.48	0.152	0.007	2.63	0.005	>20	~50
1	3.82	0.29	0.091	0.003	3.31	0.013	13.7	60
2	4.13	0.22	0.068	0.001	3.72	0.022	7.9	73
4	4.21	0.11	0.043	0.001	3.98	0.049	1.2	451
10	4.37	0.05	0.021	—	4.07	0.110	1.0	544

* 5 μM = 317.7 μg Cu l^{-1}.
† Mortality rate = $10^3 \cdot \Delta\log(f) \cdot \Delta t^{-1}$, where f = fraction surviving at time t of exposure.

Table 4. Calculated copper species activities and toxicity of 5 μM* total copper in Lake Superior water with various concentrations of pyrophosphate (pH 7.4–7.6)

Total $Na_4P_2O_7$ added (μM)	Calculated activity of copper species (μM)					Total other forms	*Daphnia* toxicity	
	Cu^{2+}	$CuOH^+$	$Cu_2(OH)_2^{2+}$	$CuCO_3$	$Cu(CO_3)_2^{2-}$		Mortality rate†	Median survival (min)
None	0.41	0.225	0.015	4.26	0.014	—‡	>20	~65
2.5	0.36	0.178	0.010	3.39	0.010	0.85 $Cu(P_2O_7)^{2-}$	17	~64
5.0	0.29	0.145	0.003	2.69	0.008	1.51 $Cu(P_2O_7)^{2-}$	14	78
10.0	0.20	0.100	—	1.91	0.006	2.33 $Cu(P_2O_7)^{2-}$	3.8	214
50.0	0.04	0.025	—	0.47	0.002	3.77 $Cu(P_2O_7)^{2-}$	0.03	>4260
500	0.00	0.001	—	0.03	—	4.13 $Cu(P_2O_7)^{2-}$	0.0	>4260

* 5 μM = 317.7 μg Cu l^{-1}.
† Mortality rate = $10^3 \cdot \Delta\log(f) \cdot \Delta t^{-1}$, where f = fraction surviving at time t of exposure.
‡ Dash indicates less than 5×10^{-10} M.

Effects of various concentrations of pyrophosphate on copper toxicity

Calculated copper species activities and toxicity of 5 μM total copper in lake water with additions of pyrophosphate from 2.5 to 500 μM are shown in Table 4. The range of added pyrophosphate bracketed that tested in the first experiment. Controls in lake water also were tested at the highest and lowest levels of pyrophosphate addition (not shown in Table 4). A single mortality occurred at 1800 min in lake water with 500 μM pyrophosphate added. Since results of the previous tests demonstrated that copper toxicity to *Daphnia* is correlated with the activity of the cationic cupric form(s), these tests were conducted to determine the feasibility of "titration" of copper with a moderately strong chelating agent (pyrophosphate) with *Daphnia* toxicity as an indication of the ionic copper remaining with incremental additions of the chelate.

Toxicity, as indicated by mortality rates or median survival time, followed the calculated decrease in Cu^{2+} with increasing pyrophosphate concentration (Table 4 and Fig. 2). A regular decrease in rate of death (and corresponding increase in median survival time) was observed up to 50 μM pyrophosphate. At this point nearly all (>99%) the copper was complexed, and the observed toxicity was negligible. At 500 μM pyrophosphate the calculated ionic Cu^{2+} activity is less than 0.001 μM, and no deaths were observed throughout the experiment (3 days).

Combining results from Tables 3 and 4, a good correlation ($r = 0.94$, $P = 0.001$) between toxicity ($1/T$) and cupric ion activity was obtained. A poor relationship was shown to $CuCO_3$ ($r = 0.47$, N.S.) or $CuP_2O_7^{2-}$ ($r = -0.61$, $P = 0.05$) activities, since toxicity was inversely related to these complexes in the two tests.

DISCUSSION AND CONCLUSIONS

Results of the present studies indicate that copper toxicity is directly related to activities of cationic

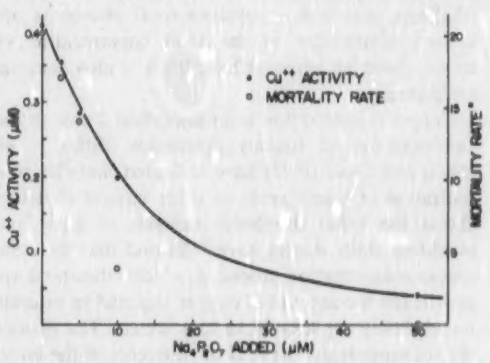

Fig. 2. Effect of various pyrophosphate concentrations on calculated cupric ion activity and copper toxicity (mortality rate) in Lake Superior water.
*Mortality rate = $10^3 \cdot \Delta\log(f) \cdot \Delta t^{-1}$, where f = fraction surviving at time t of exposure.

Cu^{2+}, $CuOH+$, and $Cu_2(OH)_2^{2+}$ (Fig. 1 and Table 2) over wide ranges of total copper, dissolved copper, and chelant concentrations. The relationship of acute toxicity to *Daphnia* and ionic copper activities was found to be linear, at least over the range tested (Fig. 1). Our results also agree with other reports indicating the very low availability and therefore toxicity, of most of the chelated or complexed forms of copper, including precipitates (Sprague, 1968; Brungs *et al.*, 1975). These and other previous investigations, however, have failed to show a direct relationship between toxicity and ionic copper activities.

Tests with various carbonate concentrations clearly demonstrate a reduction in toxicity due to carbonate complexing, and confirmed the hypothesis of Stiff (1971) indicating that this variable has a predominant effect on metal toxicity in natural waters. Our results do not support the conclusions of Shaw and Brown (1974) that $CuCO_3$ is equally as toxic as Cu^{2+}. Their interpretation, however, failed to account for differences in toxicity they observed in waters with widely differing alkalinity. Our results suggest that differences in carbonate alkalinity and shifts in carbonate equilibria caused by differences in pH may be responsible for much of the variability in copper toxicity previously reported in the literature, including that ascribed to differences in calcium hardness.

The present results also indicate, as a result of the strong influence of copper complexation on toxicity, that results of tests in various natural waters cannot be directly compared on the basis of total copper or dissolved copper measurements alone. The results from Tables 1 and 3, for example, indicate that under certain conditions toxicity may be inversely related to dissolved copper concentrations. For practical application of toxicity results, based on the total copper or dissolved copper, the chemical conditions of the test must be specified. The present results and those of Pagenkopf *et al.* (1974) indicate that retrospective interpretation of toxicity tests using equilibrium calculations is possible, if such factors as pH, carbonate alkalinity, and ortho-phosphate–total phosphate are known. Knowledge of the total concentration of strong chelating agents (Chau, 1973) is also essential for accuracy.

Copper complexation is an important factor in the determination of toxicity thresholds. Zitko *et al.* (1973) and Chau (1973) have indicated that the concentration of humic acids or other natural chelators affects the lethal threshold response of trout and plankton. Both studies have indicated that the total copper concentration producing a lethal threshold approximates the amount of copper required to saturate the chelating capacity of various waters. The present studies support this view. If the intercept of the linear relationship shown in Fig. 1(a) is assumed to approximate the lethal toxicity threshold (infinite survival) a value of 0.001–0.005 μM Cu^{2+} is obtained. This indicates that any addition of total copper resulting in cupric–ion activity above this level will result in lethality to *Daphnia*. As shown in Tables 2 and 4, Cu^{2+} activities are below this level at total copper concentrations as high as 2–5 μM when large concentrations of pyrophosphate are present; and no toxicity is observed. Therefore, toxicity thresholds based on total copper or dissolved copper measurements may be expected to vary directly with the relative strengths and concentrations of the chelating or complexing agents present in the test water, including carbonate alkalinity.

The relative toxicities of the cupric (Cu^{2+}) and copper hydroxy ($Cu(OH)_n$) ions and the effects of pH were not directly determined in the present series of tests, since pH was maintained nearly constant in each test. However, differences in the relative toxicity of Cu^{2+} ion in the three experiments indicate that increasing pH increases toxicity of the cupric ion independently of the shift in solution equilibrium. A physiological mechanism based on changes in reactivity of sulfhydryl-containing proteins or enzymes is suggested as a possible explanation for this effect. This interpretation also would account for the observations of Shaw & Brown (1974).

The approach used here, i.e., determination of the relative toxicity to calculated ionic and molecular copper complexes, offers a means for determining relative toxicities of most of the cationic transition metals and their complex forms, where sufficient knowledge exists of the stability constants and concentrations of the major complexing agents present. Furthermore, measurement of ionic activities in solutions that are acutely toxic to aquatic life is within the practical capability of recently developed analytical techniques (Zitko *et al.*, 1973), and progress should be rapid in this area in the near future.

Acknowledgements—The authors are grateful to Ms. A. Shelhon for her diligent efforts in culturing and counting the organisms used in this study. We are also indebted to Ms. S. Lappi, Ms. G. Olson, and Mr. E. N. Leonard for their analytical assistance. Special thanks are extended to Dr. Wm. Spoor for his constructive efforts in reviewing the manuscript.

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